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**OFFICE OF NAVAL RESEARCH  
FINAL PROGRESS REPORT**

**Grant Number:** N00014-02-1-0329

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**Employer Identification #:** 53-0204-660

**Project Title:** Cellular and Molecular Responses to Depressed Metabolism and Low Temperature

**Project Award:** \$10,000

**Project Duration:** 12 months July 1, 2002 to June 30, 2003

**Introduction**

The purpose of this proposal was to provide support for a symposium organized by Hannah Carey and Gregory Florant entitled "Cellular and Molecular Responses to Depressed Metabolism and Low Temperature." The symposium is one of several symposium scheduled for presentation during an American Physiological Society conference entitled "The Power Of Comparative Physiology: Evolution, Integration And Applied", scheduled for August 24-28, 2002 in San Diego, California.

The overall goal of the APS Intersociety Meeting was to bring together comparative biologists who utilize a diversity of approaches including molecular, cellular, organ, and organismal physiology/biochemistry, functional morphology, biomechanics and biophysics, ecology and evolutionary biology to understand physiological processes and traits. The aim of the symposium supported by the ONR was to present recent advances in gene expression and cellular signaling pathways that mediate adaptive responses to low body temperatures and metabolic depression. Several of the talks focused on the use of new approaches from genomics and proteomics to elucidate pathways that permit survival during altered thermal and metabolic states. The symposium contained presentations on organisms ranging from lower vertebrates to mammals in order to identify common mechanisms as well as alternative pathways of adaptation.

## Body

Since its founding in 1887 by five noted scientists, the American Physiological Society (APS) has been devoted to fostering basic and applied scientific information. Through these activities, the Society has grown to approximately 10,600 members and continues to make major contributions to the progress of science and the advancement of biological and biomedical knowledge.

The Society sponsors one general scientific meeting in the spring that is devoted to the dissemination of newly acquired scientific information. The Spring Experimental Biology Meeting is held with other societies that are members of the Federation of American Societies for Experimental Biology (FASEB). The April 2001 meeting attracted over 11,000 scientists and exhibitors with physiological scientists contributing more than 2,600 papers in 175 sessions. In addition, there were 53 APS-sponsored symposia involving some 250 speakers and 16 invited lecturers.

The Society also regularly sponsors up to two other meetings, called the APS Conferences, each year. In 2002, APS sponsored two conferences. From February 20-23, 2002, APS held a conference in San Francisco entitled "Physiological Genomics of Cardiovascular Disease: From Technology to Physiology" and in August the Society sponsored this conference entitled "The Power Of Comparative Physiology: Evolution, Integration And Applied."

The Society is also a major publisher of journals and books on physiology. The journals of the Society include the *American Journal of Physiology*, the *Journal of Applied Physiology*, the *Journal of Neurophysiology*, and *Physiological Reviews*. All of the journals are currently available online with the assistance of HighWire Press. The Society, in conjunction with the International Union of Physiological Sciences, also publishes a trends-type journal, entitled *News in Physiological Sciences (NIPS)*. The Society recently launched a new journal, *Physiological Genomics*, which appears online prior to print.

In planning for this conference, the organizers were drawing upon the American Physiological Society's strong commitment to comparative physiology. In the Fall of 1990 in Orlando, Florida, the APS sponsored a meeting, largely organized by the Comparative Section centered on the theme "*In Search of Physiological Principles - The Use of Animal Diversity and Novel Technology*." Guest societies at this meeting included Society for Integrative and Comparative Biology (SICB, formerly known as American Society of Zoologists), Society for Experimental Biology (SEB), Canadian Society of Zoologists (CSZ) and the Comparative Respiratory Society. Based on the success of the Orlando meeting, it was clear that the APS provided an important service to the widely dispersed community of comparative physiologists and it was the hope of the comparative section that such a meeting would be sponsored on a regular basis (every four years).

In the Fall of 1994 in San Diego, California, the APS continued its sponsorship of a large comparative meeting, this time based on the theme "*Regulation, Integration, Adaptation: A Species Approach*." Guest societies at this meeting included SICB, SEB, CSZ and the German Society of Zoologists. The combined success of Orlando and San Diego indicated that the APS had assumed the leadership role in providing a home for and defining the future of comparative physiology.

The scheduling of this conference by APS reasserted APS' leadership role in defining comparative physiology as we enter the 21<sup>st</sup> century.

Comparative biology, through its diversity of investigative approaches, is an extremely powerful paradigm for studying physiology. It is the only approach to physiological research that, by its very nature, often seeks to understand physiological processes and traits over several different time domains. These domains range from investigating the proximal details of physiologic mechanisms to investigations that aim to understand and gain insights into ultimate causation, i.e. the evolutionary or adaptive significance of a physiological process or trait. Consequently, comparative biology utilizes a diversity of approaches including molecular, cellular, organ, and organismal physiology/biochemistry, functional morphology, biomechanics and biophysics, ecology and evolutionary biology. Within the past 10 years, comparative physiology has undergone rapid changes resulting from the incorporation of a variety of new tools and technologies. These include the many new and powerful tools of molecular biology, the use of remote sensing and physiological monitoring technologies, advances in microelectronics and computers and the rigorous application of evolutionary theory. Singularly and in combination these approaches have resulted in a rethinking of many long held concepts and constructs in comparative physiology as well as the development of new syntheses.

The meeting highlighted the accomplishments that have occurred since the last large comparative meeting and more importantly, provided a forum to showcase new directions and approaches.

Guest societies included the Society for Integrative and Comparative Biology (formerly American Society of Zoologists), Society for Experimental Biology (SEB, UK), Canadian Society of Zoologists (CSZ, Canada), German Society of Zoologists (GSZ, Germany) and the Australian Society of Comparative Biochemistry and Physiology.

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The conference was held over four-days, starting on Saturday evening and ending on Wednesday evening. The full schedule of sessions, including a listing of lectures, symposia, and poster presentations is included in the form of an appendix.

## **Reportable Outcomes and Conclusions**

### **Symposium Outcomes - Cellular and Molecular Responses to Depressed Metabolism and Low Temperature**

#### **Symposium Organizers:**

##### **Hannah V. Carey, Ph.D.**

Department of Comparative Biosciences  
University of Wisconsin School of Veterinary Medicine  
Madison, WI

##### **Gregory Florant, Ph.D.**

Department of Biology  
Colorado State University  
Fort Collins, CO

The overall goal of the APS Intersociety Meeting was to bring together comparative biologists who utilize a diversity of approaches including molecular, cellular, organ, and organismal physiology/biochemistry, functional morphology, biomechanics and biophysics, ecology and evolutionary biology to understand physiological processes and traits.

The aim of the symposium supported by the ONR was to present recent advances in gene expression and cellular signaling pathways that mediate adaptive responses to low body temperatures and metabolic depression. Several of the talks focused on the use of new approaches from genomics and proteomics to elucidate pathways that permit survival during altered thermal and metabolic states. The symposium contained presentations on organisms ranging from lower vertebrates to mammals in order to identify common mechanisms as well as alternative pathways of adaptation.

The symposium consisted of seven primary speakers and 2 shorter presentations that were solicited from abstracts that were contributed to this particular session from meeting participants. Dr. Carey opened the symposium with some introductory remarks that highlighted the breadth and depth of the topics that would be covered in the session. A synopsis of each of the speakers' presentation follows.

#### **1. "Acquired cold tolerance - moving from genes to a genomic overview"**

Andrew Cossins, Ph.D., The Consortium for Post-Genome Sciences, University of Liverpool

Dr. Cossins first outlined the identification by his laboratory in 1996 that the delta-9 desaturase gene is responsible for transcriptional and translational regulation of membrane function during cold adaptation in carp. He then described how genetic and molecular studies

have been subsequently used to understand how animal cell membranes are altered by both diet and temperature to permit efficient function in altered environmental conditions. Dr Cossins's presentation concluded with a general discussion of the power of genomic tools to increase insight into the adaptive responses that animals display to maintain function during altered states.

**2. "Gene expression profiling of aging and its retardation by caloric restriction"**

**Tomas Prolla, Ph.D.**, Department of Genetics, University of Wisconsin, Madison

Dr. Prolla described the pioneering studies from his laboratory on gene expression profiling in aging mammals (rodents and primates) and how this can be altered by caloric restriction. Many genes activated during aging in animals fed *ad libitum* are indicative of the cellular response to stress, particularly oxidative stress, and these stress indicators are lessened when animals consume diets that provide 40% of ad libitum calorie levels. As pointed out by Dr. Prolla, although gene array and similar molecular techniques are providing enormous amounts of information in the aging field, physiologically-based studies are ultimately required to understand the significance of the information as it relates to whole organisms. Later during the question period, Dr. Prolla also noted that lessons learned from non-traditional organisms such as hibernators will also be very useful in understanding the adaptive significance of the cellular and molecular changes that are induced by aging and caloric restriction.

**3. "Molecular determinants of the hibernating phenotype"**

**Sandra Martin**, Cellular and Structural Biology, Univ. of Colorado Health Sciences Center

Dr. Martin began with a discussion of the rationale that mammalian hibernation is most likely an adaptation involving genes already existing in the mammalian genome and not novel genes that have arisen specifically in species that hibernate. That is, differential expression of genes that are also present in the human genome through transcriptional, translational and post-translational modifications are likely responsible for the hibernating phenotype. Dr. Martin then outlined the evidence that rather than adaptation for improved function in the cold, the key molecular adaptations of mammalian hibernators may lie in their ability to exploit low temperatures to depress most aspects of biochemical function, then rewarm without damage. She concluded with new studies on methods of proteomic determination of altered gene expression in hibernators.

**4. "Mammalian hibernation through the eyes of mRNA expression profiling"**

**Matthew Andrews**, Department of Biology, University of Minnesota-Duluth

Dr. Andrews outlined work in his laboratory that is identifying, through differential gene expression studies, the physiological and biochemical modifications that occur to metabolic pathways in hibernators that permit the well-known switch from carbohydrate to fatty acid metabolism. He also described new studies in his lab that are revealing changes in neuroendocrine signaling pathways that may be responsible for the central control of metabolic depression as animal enter and maintain the torpid state.



**5. “Insulin signaling pathways in mammalian hibernators”**

**Gregory Florant**, Colorado State University

Dr. Florant’s presentation focused on new work on the cellular basis of insulin regulation in hibernators. For example, the peripheral insulin resistance characteristic of mammalian hibernators shortly before they enter torpor has been known for some time, but how this resistance is induced at the level of cellular signaling pathways has been obscure. Work in the Florant laboratory had identified the protein Akt (also known as protein kinase B) as a potential key regulator in this process. Interesting, Akt also plays a role in cell survival in other systems through its actions as an anti-apoptotic protein.

**6. “Integration of hypometabolism and hypoxia via ROS signaling”**

**Paul Schumacker**, Department of Medicine, University of Chicago

Dr. Schumacker discussed his work using isolated cardiomyocytes to identify signaling mechanisms by which cells adaptively respond to hypoxic conditions. Such studies are revealing how hypoxia and hypometabolism are coordinated through signaling pathways involved reactive oxygen species to respond to low oxygen conditions in the environment. The transcription factor hypoxia-inducible factor –1 (HIF-1) plays a critical role in mediating many of the adaptive responses to hypoxia including induction of VEGF and erythropoietin, proteins that help increase vascular supply of oxygenated blood to tissue beds.

**7. “Stress-induced signaling pathways associated with depressed metabolism and low temperature”**

**Hannah Carey**, Department of Comparative Biosciences, University of Wisconsin

Dr. Carey discussed studies from her own and other laboratories that are revealing how hibernating mammals appear to utilize moderate degrees of oxidative stress as part of the adaptation to greatly altered metabolism and physiology that is experienced each year during the annual cycle. Using responses of gut tissue in ground squirrels as an example, she described how nutritional and oxidative stress in the intestine lead to changes in redox balance and induction of stress-activated chaperone proteins and transcription factors during the hibernation season. She also described new work on the relationship between cell proliferation and apoptosis in intestinal epithelial cells during hibernation. Dr. Carey concluded by emphasizing how efforts to exploit hibernation for improvements in biomedicine should focus on cellular and molecular mechanisms that allow hibernators to tolerate physiological conditions that are considered highly stressful for non-hibernating species, such as humans. Questions after this presentation from Drs. Prolla and Schumacker highlighted the similarities that are being found between the hibernating phenotype and aging/caloric restriction studies as well as mechanisms of hypoxia and hypometabolism identified at the cellular level.

The symposium concluded with two shorter talks chosen from abstracts submitted to the conference that were relevant to this session.

**Dr. Timothy O’Connor** (City University of New York, now at the Dept of Molecular Medicine, Cornell Medical College) presented a talk entitled “Enhanced antioxidant activity in

the longest-living rodent species (*Heterocephalus glaber*)". This presentation summarized studies that examined several hypotheses that were developed to account for the remarkable longevity of these small mammals in terms of physiological and biochemical mechanisms. The presentation very nicely tied together issues raised in Dr. Prolla's talk and those from Dr. Carey's talk related to oxidative stress. Dr. O'Connor was an Assistant Professor of Biology at City College of New York, who recently moved to the

**Dr. Nancy Pruitt** (Colgate University) talked on "Evidence for a cryoprotective protein in freeze-tolerant larvae of the goldenrod gall fly, *Eurosta solidaginis*". Dr. Pruitt work in her laboratory that is characterizing tolerance proteins in gall fly larvae that are responsible for the striking ability of the animals to withstand freezing temperatures as part of their normal life history.

### **Summary:**

This symposium clearly was well-received by the audience, which averaged about 75-100 people throughout the 4-h session. The breadth of symposium complimented very well its focus on physiological adaptations to altered metabolism and body temperature. As the symposium proceeded, several key themes and commonalities emerged that in some cases were anticipated and, in other cases, not initially recognized. For example, empirical studies with hibernating mammals in the past have indicated a relationship at the whole organism level between longevity and metabolic depression. However, Dr. Prolla's presentation on caloric restriction and aging in rodents and primates revealed several features at the cellular and molecular levels that are also characteristic of other models of metabolic depression discussed during the session, yet were not appreciated until now. (In fact, it is likely that new collaborations among some of the speakers will emerge due to interactions that took place during and after this session.) The questions from the floor after many of the talks further demonstrated the common themes that emerged from the session. The value of this session was also evident during symposia held on subsequent days of the conference. Several speakers in subsequent sessions referred back to presentations from our symposium as they discussed their respective topics. Thus, the scheduling of this session on the first day of the conference helped make the meeting as a whole particularly integrative, informative and exciting. Overall, the APS Intersociety Meeting was a resounding success and the support of the Office of Naval Research was very much appreciated.

### **Meeting Outcomes - The Power of Comparative Physiology: Evolution, Integration, and Application**

The APS returned to the Town & Country Resort and Conference Center in San Diego for its third APS Intersociety meeting focusing on comparative physiology entitled "*The Power of Comparative Physiology: Evolution, Integration, and Application*". The meeting, held August 24-28, included six guest societies: the Society for Integrative and Comparative Biology (SICB), Society for Experimental Biology (SEB), Canadian Society of Zoologists (CSZ), German Society of Zoologists (GSZ), the European Society of Comparative Physiology and Biochemistry (ESCPB), and the Australian & New Zealand Society of Comparative Biochemistry and Physiology (ANZSCBP).

As Chair of the Organizing Committee, **James Hicks** (University of California, Irvine) created a 4-day meeting that incorporated five plenary lectures, 19 symposia and 21 poster sessions. Presenters covered topics as wide-ranging as: regulation, renal function, genetics, hypoxia, integration, motor function, metabolism, neurophysiology, microarrays, homeostasis, muscle physiology, environmental physiology, diving, physiological evolution, host-parasite interactions, artificial muscles and robots, hypoxia, developmental physiology and plasticity. The organisms studied were also quite diverse, ranging from invertebrates (like the fruit fly and cabbage looper) on up the evolutionary scale to frogs, fish, reptiles, birds and mammals.

The meeting attracted 554 registrants—31% (177) were female and 35% (196) represented young scientists; including 54 postdoctoral and 142 student registrants. 22% (123) were members of APS or one of the 6 guest societies; 10% (58) were nonmembers; 28% (156) were invited speakers or organizers. Of the total registrants, 19% (105) worked outside The Americas, 3% (18) in US government labs and 1% (8) in industry.

Registration Type	Total	Percent
Member (APS or Guest Society)	123	31%
Retired Member	5	>1%
Nonmember	58	10%
Postdoctoral	54	9%
Student	142	25%
Invited Speaker or Organizer	156	28%
Nonscientist Guest of Registrant	15	2%
Undergraduate	1	>1%
	<b>554</b>	

The meeting agenda was arranged to feature a morning plenary lecture, followed by 4-5 concurrent symposia, which were then followed by unopposed poster sessions. The poster sessions were designed to maximize interaction among participants and featured beer, wine and light snacks. These *Poster Socials*, were sponsored each day by one of the top four journals publishing comparative research: Sunday was sponsored by *Comparative Biochemistry and Physiology*, Monday by *Physiological and Biochemical Zoology*, Tuesday by the *Journal of Experimental Biology*, and Wednesday was sponsored by the *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*.

In addition to the scientific sessions, several social activities were offered to attendees. The Opening Reception on Saturday evening was an informal reception held poolside at the famed Town & Country's Tiki Hut; Monday featured a special-purchase dinner at the Scripps Institute of Oceanography's Birch Aquarium, and; the last night, Wednesday, featured the Scholander Award Banquet and Lecture. The Scholander Award lecture was presented by **Barbara Block** (Hopkins Marine Station, Stanford University) entitled "*The Fire Inside: Saving Atlantic Bluefin Tuna*".

Three awards for best abstract presentation by a graduate student were presented during the Scholander Banquet. Recipients and their respective awards were: **The Society of Integrative and Comparative Biology Young Investigator Award** presented to **John Zehmer**, Arizona State University for his presentation entitled "*Plasma membrane rafts of rainbow trout are subject to thermal acclimation*"; the **Society for Experimental Biology Young Investigator Award** presented to **Scott D. Kirkton**, Arizona State University for his presentation entitled "*Oxygen delivery problems may reduce jumping performance in larger locusts*", and; the **Scholander Award**, sponsored by the **APS Comparative Physiology Section**, presented to **Todd E. Gillis**, Simon Fraser University for his presentation entitled "*Sequence mutations in teleost cardiac troponin C that are permissive of cardiac function at low temperatures*". Each awardee received a cash prize and a one year complimentary subscription to the journal published by the sponsoring society.

From the outset, the inclusion young investigator participation was very important to the organizing committee who therefore designed a travel award program for graduate students and postdoctoral fellows. 55 travel grants were provided totaling over \$30,000. Travel awardees that met application guidelines received partial travel reimbursement, complimentary registration and a ticket to the Birch Aquarium dinner event.

There were eleven recipients of the APS Porter Physiology Development Committee's Minority Travel Fellowship Award, supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institute of General Medical Sciences (NIGMS). The fellowship provides reimbursement of travel expenses and each recipient is matched with an APS member attending the meeting who offers guidance and makes introductions to other scientists. Recipients were: **Lee A. Aggison, Jr.**, Stillman College; **Thomas F. Gallegos**, New Mexico State University; **Vallie M. Holloway**, Loyola University Medical Center; **Rafael Alejandro Leos**, New Mexico State University; **Marcy K. Lowenstein**, Florida International University; **Rudy M. Ortiz**, University of California, Santa Cruz; **Elizabeth S. Quintana**, New Mexico State University; **Luciana Oliveira Santos**, University of Utah; **LaTonia Marie Stiner**, Wright State University; **Vanessa I. Toney**, Brown University; and **Ruth A. Washington**, Stillman College.

In all, 291 abstracts were programmed into poster sessions. Of these 36% (107) were represented by female presenters and 23% (68) were from countries outside The Americas. Researchers working in industry comprised 1% (4); those from US government labs also represented 1% (4) of the total submissions.

The Society wishes to thank the members of the Intersociety Meeting Organizing Committee: **James Hicks**, Chair (University of California, Irvine), **Albert Bennett** (University of California, Irvine), **Barbara Block** (Hopkins Marine Station, Stanford University), **Steven C. Hand** (Louisiana State University, Baton Rouge), **Donald C. Jackson** (Brown University) and, **Stephen C. Wood** (VA Medical Center, Nashville).

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**References**

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